AUTOMATED 3D RECONSTRUCTION OF THE NORMAL AND CIRRHOTIC RAT HEPATIC MICROVASCULATURE USING CONFOCAL MICROSCOPY: A FEASIBILITY STUDY

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1. INTRODUCTION

Liver cirrhosis is a chronic disease of the liver, comprising a wide spectrum of pathological characteristics affecting the hepatic architecture and function. To date, little is known about the hemodynamic consequences caused by cirrhosis, especially at the microscopic level. In order to analyze the morphology and conduct computational flow simulations, accurate 3D reconstructions of the hepatic microcirculation are indispensable [1]. In this study, we aim to combine immunohistochemistry and confocal laser microscopy, enabling us to acquire detailed 3D geometrical data of the liver microarchitecture.

2. METHODS

After whole animal perfusion fixations with 4% paraformaldehyde, normal and cirrhotic livers were resected from male Wistar rats. Subsequently, immunohistochemistry was applied to 100 µm thick slices by staining the endothelial cells with the monoclonal antibody RECA (Rat Endothelial Cell Antigen) and the fluorescent cyanine dye Cy3. 2D image stacks were recorded with a confocal microscope at a resolution of 0.61 µm. To visualize the liver sinusoids in 3D, the obtained datasets were automatically processed and segmented with in-house developed software using ITK, VTK and Qt libraries (see Fig. 1a-b).

3. RESULTS

The results indicate that automatic reconstruction of the hepatic vascular network is feasible for normal and cirrhotic livers (see Fig. 1c). Currently, the visualization depth is limited to 40 - 50 µm for rat livers. Several techniques (stitching, clearing, bidirectional imaging etc.) are being explored to increase the imaging depth.

4. CONCLUSION

The aforementioned pipeline provides a useful tool to accurately reconstruct the 3D architecture of the hepatic microcirculation, which may lead to new insights in the altering morphology of liver cirrhosis. In addition, the technique is not restricted to the vascular network, but can be extended to the simultaneous staining of the biliary network as this ramifying tree and its respective functions are also affected by cirrhosis.

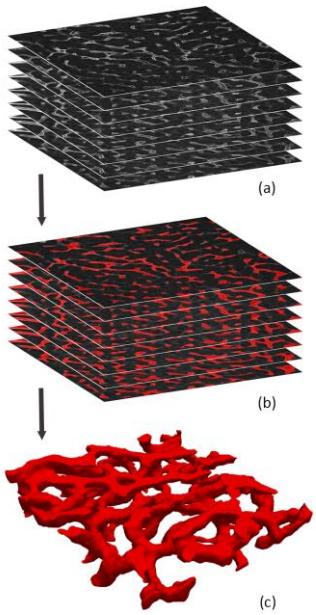

Fig. 1: (a) Raw dataset of a RECA-stained cirrhotic rat liver slice, obtained with confocal microscopy. (b) Automatically processed and segmented dataset indicating the vascular network. (c) Anatomically correct 3D reconstruction of the cirrhotic microcirculation of a rat liver.

References

[1] Peeters, G. et al. A multilevel modeling framework to study hepatic perfusion characteristics in case of liver cirrhosis, *J. Biomech Eng*, 137(5), 2015.